# Domain Analysis of IL-1 Receptor-Associated Kinase 1

Sim-Hui Tee

Multimedia University, Persiaran Multimedia 63100 Cyberjaya, Selangor, Malaysia

shtee@mmu.edu.my

***Abstract***

There are four IL-1 Receptor-Associated Kinase (IRAK) family members in mammals, one of which is IRAK1. IRAK1 interacts with IRAK4, which is associated with MyD88 adaptor protein upon ligand stimulation. This research aims to study the domains of IRAK1, understanding of which is important to yield insights into the functional role of IRAK1 in the pathogen sensing mechanism. The results obtained from this study may guide further research in the immunotherapy studies on the Toll-Like Receptor (TLR) signaling pathway.

***Keywords***

*Bioinformatics; Gene; Protein; IRAK1; Domain Analysis; Kinase*

## Introduction

IL-1 Receptor-Associated Kinase 1 (IRAK1) is an important kinase that is implicated in the pathogen sensing signaling pathways in the immune system. IRAK1 has been reported (Takeshita and Ishii 2008) to act as a triggered kinase in the detection of CpG DNA by Toll-like receptor 9 (TLR9). Besides, it was found that IRAK1 plays a significant role as a signal amplifier that would culminate in the activation of NF-κB (O'Neill 2006). Although IRAK1 was not found to involve in the inflammasome signaling pathway, IRAK4, a member from the same kinase family as IRAK1, was reported to assemble with MyD88 in the NLRP3 inflammasome activation (de Nardo and Latz 2011).

In the TLR signaling pathway, MyD88 adaptor protein is associated with TLRs via Toll-IL-1 receptor (TIR) domain interactions. The bound TLR-MyD88 complex then recruits IRAK4 by binding to its death domain. The activated IRAK4 further phosphorylates IRAK1, leading to the activation of downstream Traf-6, Tak-1, inhibitor of NF-κB (IKK), IRF7, and transcription factor NF-κB (O'Neill 2006; Blasius and Beutler 2010). Activated NF-κB will translocate into nucleus and regulate the expression of type I interferon production. The appropriate level of type I interferon is critical not only in the immune defense against pathogens (Axtell and Steinman 2008); its aberration will have enormous impacts on the autoimmunity (Ewald and Barton 2011).

Structural insight of the proteins is important for the disclosure of the function. Besides, structural studies also provide clues on the interaction between ligands and their receptors (Wei et al. 2011). In the field of protein science, structural studies normally are carried out by electron microscopy (Nasertorabi et al. 2011), NMR techniques (Wang et al. 2011), and crystallography (Piotrowski et al. 2009). These approaches are complementary in the efforts to better understand the protein structure and function (Hunter et al. 2011). Despite the widespread use of physical approaches, computational approach has also been used increasingly due to its efficiency in the biological research. To date, computational analysis of the biological processes and entities has been applied in various fields, such as cellular pathways (Zhang et al 2009), cytoskeleton studies (Dodson and Dimitrakopoulos 2010), protein structure (Kim 2004; Arakaki et al. 2004; Hinsen 2008), RNA (Levenkova et al. 2004; Jonikas et al. 2009; Muller 2005), viral studies (Zhou et al. 2005; Itakura et al. 2010), immunogenetics (Massari et al. 2012), signalling pathways (Xu and Wong 2008; Fujarewicz et al. 2007; Lai et al. 2009), cellular energies (Andronescu et al. 2010), oncology (Han 2010; Wang et al. 2007; Oh and Gao 2011; Wang et al. 2011), molecular docking (Axenopoulos et al. 2011; Zhang et al. 2005; Hussain et al. 2010; Teixeira et al. 2011), protein folding (He et al. 2009; Roche et al. 2011; Csaba and Zimmer 2010), carbohydrate structure (Ranzinger et al. 2011), and drug discovery (Sato et al. 2012; Kushwaha and Shakya 2010).

In this research, a computational approach has been adopted to analyze the domains of IRAK1 protein. Both active site and substrate binding site of IRAK1 were studied in detail. The best template for IRAK1 was predicted in this study. The insights of this research may guide further research in the

immunotherapy studies of IRAK1-implicated pathways.

## Methods

The nucleotide and amino acid sequence of IRAK1 were retrieved from National Center for Biotechnology Information (NCBI). We used PSI-BLAST (Altschul et al. 1997) for distant homology recognition. Cn3D version 4.3 was used to simulate the domains of the protein. FFAS03 method (Jaroszewski et al. 2005) has been used for the calculation of the confidence score for the structural domains of IRAK1. Sequence alignment between target protein and template protein was carried out using SCWRL (Krivov et al. 2009) and JACKAL modelling approach. Lastly, Pcons server (Lundstrom et al. 2001) was used to validate the structure of IRAK1.

## Results and Discussion

Sequence alignment was performed on the active site and substrate binding site of IRAK1. Fig. 1 illustrates the alignment for active site, with the domain highlighted in yellow. All of the proteins shown in Fig. 1 are kinases. Fig.2 illustrates the alignment for substrate binding site.


FIG. 1 SEQUENCE ALIGNMENT OF THE ACTIVE SITE


FIG. 2 SEQUENCE ALIGNMENT OF THE SUBSTRATE BINDING SITE.

The abbreviation of the molecules presented in Fig. 1 and Fig. 2 is as such: 1F3M_C is Chain C, Crystal Structure of Human Serine/Threonine Kinase PAK1; 1L3R_E is hain E, Crystal Structure of A Transition State Mimic of The Catalytic Subunit of Camp-Dependent Protein Kinase; 1FGI_A is Chain A, Crystal Structure of The Tyrosine Kinase Domain of Fibroblast Growth Factor Receptor 1 In Complex With Su5402 Inhibitor; 1T4H_B is Chain B, Crystal Structure of The Kinase Domain of Wnk1; gi 68125618 is protein kinase (Leishmania major strain Friedlin); gi 48428484 is Serine/threonine-protein kinase PINK1; gi 78045619 is protein kinase (Xanthomonas campestris pv. vesicatoria str. 85-10); gi 50057639 is Protein kinase, (Paramecium tetraurelia); gi 125291 is Serine/threonine-protein kinase HAL4/SAT4.

From the sequence alignments, it was found that the extent of sequence identity is low, hence it is quite unlikely to identify a meaningful putative functional relationship, which is one of the objectives of the sequence alignment (Batzoglou 2005). The simulated domains are given in Fig. 3 (Ball and Stick model) and Fig. 4 (worms model), respectively. Homology relationship can be accurately recorded by the structural similarity, due to the fact that structural similarity is preserved beyond sequence similarity. Understanding of the three-dimensional structure of a protein may facilitate the understanding of the function of each domain (Vogel et al. 2004).

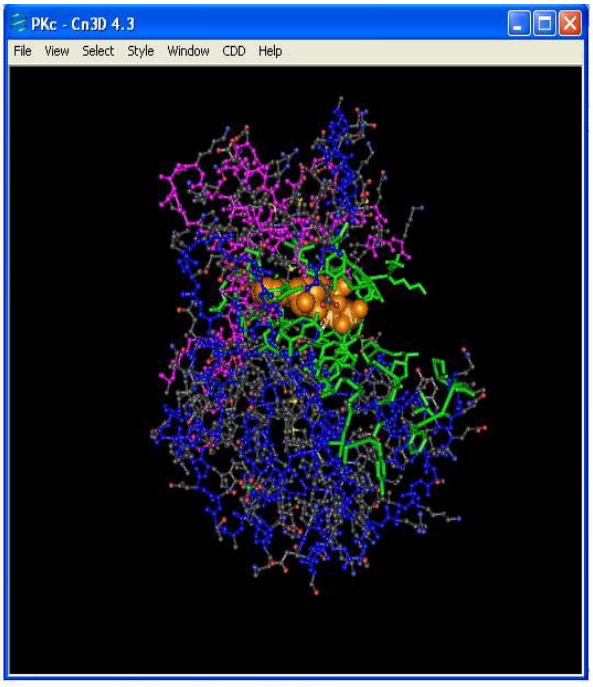


FIG. 3 PROTEIN DOMAINS (BALL AND STICK MODEL)

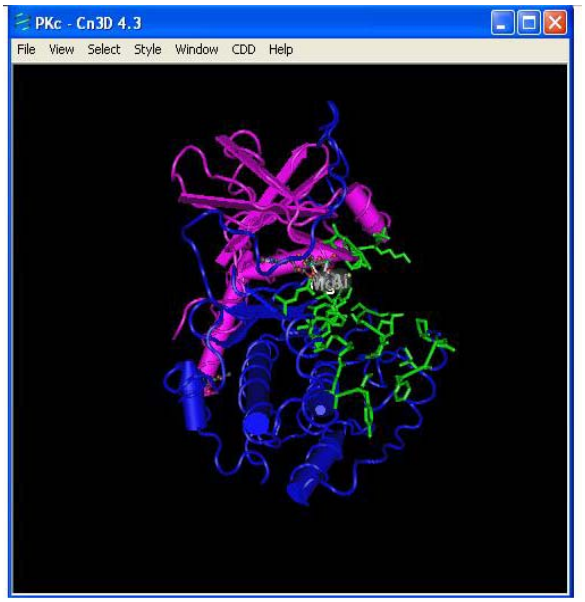


FIG. 4 PROTEIN DOMAINS (WORMS MODEL)

FFAS03 method (Jaroszewski et al. 2005) has been used for the calculation of the confidence score for the structural domains of IRAK1. Table 1 lists the top 10 best matched templates with IRAK1. It is apparent that 2nru protein is the most probable protein template that matches the structure of IRAK1.

TABLE 1 BEST MATCHED PROTEIN TEMPLATES FOR IRAK1 (USING FFAS03)

| Confidence Score | Targets | Protein length (aa) | Description |
|---|---|---|---|
| -40.40 | 2nru | 307 | Interleukin-1 receptor-associated kinase 4 |
| -40.00 | 3hgk | 327 | Protein kinase |
| -37.70 | 3t18 | 349 | BRASSINOSTEROID INSENSITIVE 1-associated rece |
| -30.80 | 2qlu | 314 | Activin receptor type IIB |
| -30.80 | 3q4t | 322 | Activin receptor type-2A |
| -30.40 | 3p86 | 309 | Serine/threonine-protein Kinase CTR1 |
| -30.10 | 4f0f | 287 | Serine/threonine-protein Kinase roco4 |
| -29.80 | 3g2f | 336 | Bone morphogenetic Protein receptor type-2 |
| -29.10 | 3dtc | 271 | MAPK |
| -28.00 | 3s95 | 310 | LIM domain kinase 1 |

Based on the confidence score listed in Table 1, we identified 2nru as the potential protein template to model the structure of IRAK1. When there is a clear sequence relationship between a target protein and a

known structure, structural templates can be used to yield an accurate core model (Moult 2005). We modelled IRAK1 against 2nru to find the identical residues. Such approach has been used in the experiment to study the glycosylase domain of DML proteins (Ponferrada-Marín et al. 2011). In this study, IRAK1 was aligned with 2nru (aa: 15) starting at amino acid position 208. Fig. 5 demonstrates the identical residues of both strands, as highlighted in red.


FIG. 5 PROTEIN STRUCTURE MODELLING

To validate our results, we used Pcons server to predict the structure of IRAK1. Table 2 lists the predicted matches.

TABLE 2 BEST MATCHED PROTEIN TEMPLATES FOR IRAK1 (USING PCONS)

| Rank | Score | Targets |
|---|---|---|
| 1 | 3e-98 | 2nru |
| 2 | 3e-90 | 1vjy |
| 3 | 4e-89 | 2b7a |
| 4 | 4e-87 | 2fb8 |
| 5 | 4e-86 | 2eva |
| 6 | 4e-86 | 1yvj |
| 7 | 1e-80 | 1sm2 |
| 8 | 9e-78 | 2dq7 |
| 9 | 2e-77 | 1k2p |

Comparing Table 2 with Table 1, 2nru is the best template yielded using FFAS03. Besides, it is also validated by Pcons server. It has four chains (A, B, C, D) with a structure weight of 140266.73. The crystal structure of this protein in the phosphorylated form has been reported by Wang et al. 2006. Further evidence from the bound protein assembly suggests that both IRAK1 and 2nru are assembled with MyD88 in the similar way (Honda et al. 2004). Thus, 2nru represents the best matched protein template for IRAK1.

## Conclusion

IRAK1 is one of the important kinases in the pathogen sensing pathway. It is found to bind to the MyD88 adaptor protein in the signaling cascade, which is leading to the activation of NF-κB transcription factor. The domain study of IRAK1 is important for the disclosure of its functional role in the cytokine production and subsequent recruitment of lymphocytes to the sites of inflammation. In this study, we found that the structure of IRAK1 is closely resembled to 2nru, which suggests that IRAK1 and IRAK4 are highly homologous. Because both IRAK1 and IRAK4 have domain similarity to MyD88, it is of interest to study other potential MyD88-bound proteins which are yet to discover. The domain analysis of IRAK1 may provide insights into the therapeutic strategies that target MyD88 signaling pathway.

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